A Large Family of Divergent Drosophila Odorant-Binding Proteins Expressed in Gustatory and Olfactory Sensilla

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ABSTRACT

We identified a large family of putative odorant-binding protein (OBP) genes in the genome of *Drosophila melanogaster*. Some of these genes are present in large clusters in the genome. Most members are expressed in various taste organs, including gustatory sensilla in the labellum, the pharyngeal labral sense organ, dorsal and ventral cibarial organs, as well as taste bristles located on the wings and tarsi. Some of the gustatory OBPs are expressed exclusively in taste organs, but most are expressed in both olfactory and gustatory sensilla. Multiple binding proteins can be coexpressed in the same gustatory sensillum. Cells in the tarsi that express OBPs are required for normal chemosensation mediated through the leg, as ablation of these cells dramatically reduces the sensitivity of the proboscis extension reflex to sucrose. Finally, we show that OBP genes expressed in the pharyngeal taste sensilla are still expressed in the *poxneuro* genetic background while OBPs expressed in the labellum are not. These findings support a broad role for members of the OBP family in gustation and olfaction and suggest that *poxneuro* is required for cell fate determination of labellar but not pharyngeal taste organs.

ANIMALS are dependent on chemical senses for foraging, reproduction, and avoidance of noxious environments. Like other insects, *Drosophila melanogaster* detect volatile chemical signals with neurons located on their antenna and maxillary palps, while tastants are detected by contact chemoreceptors distributed in the mouth area and broadly over the surface of the animal (Stocker 1994; Smith 2001). Drosophila adults have ~2000 chemosensory neurons that reside within segregated compartments called sensilla. Each sensillum is a hair-like, hollow, fluid-filled structure containing the dendrites of olfactory neurons bathed in sensillum lymph. A single sensillum contains the dendrites of between one and four olfactory neurons. Odorants enter the sensilla through pores or grooves in the cuticular wall, dissolve in the sensillum lymph, and activate olfactory neurons.

Olfactory sensilla are located on the distal segment of the antenna and on the maxillary palps. These sensilla have three distinguishable morphologies, the basiconic, trichoid, and coeloconic sensilla (reviewed in Stocker 1994). Recently a fourth intermediate class has been described that has features of both trichoid and basiconic sensilla (Shanbhag *et al.* 1999). All classes of olfactory sensilla detect odorants (Siddiqi 1987; Clyne *et al.* 1997) and the functional significance of the morphological differences is unknown. Functionally, different sensilla respond to different odorants (Siddiqi 1987; Clyne *et al.* 1997; De Bruyne *et al.* 2001).

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Gustatory sensilla are similar to olfactory sensilla, with hair-like projections containing chemosensory neurons and sensillum lymph, but are morphologically distinct and widely dispersed over the surface of the animal. Taste bristles (TBs) are gustatory sensilla that have a terminal pore where tastants are thought to enter and have a split lumen connected at the tip. One side contains the gustatory neuron dendrites of two to four chemosensory neurons and the other lumen contains only sensillum lymph (Morita 1992). Taste bristles located on the labellum are present in three rows. TBs are also present on the legs and anterior wing margins. Pharyngeal taste organs, including the labral sense organ and the dorsal and ventral cibarial sense organs, also contain sensilla that are thought to sample food within the esophagus.

Odorant-binding proteins (OBPs) are present in the olfactory systems of both vertebrate and invertebrate animals, but these gene families are not related. The invertebrate odorant-binding proteins found in insects are not lipocalin family members like their vertebrate counterparts, but are composed of proteins encoded by a distinct gene family. X-ray crystal structure data obtained from the vertebrate and invertebrate OBPs reveals no structural relationship between the two groups (Bianchet *et al.* 1996; Sandler *et al.* 2000). Vertebrate OBPs bind odorants at the interface of a dimer (Bianchet *et al.* 1996) while the insect OBPs appear to bind ligand as monomers (Sandler *et al.* 2000).

Members of the invertebrate odorant-binding protein family described to date are olfactory-specific molecules secreted from nonneuronal support cells into the sensillum lymph of subsets of olfactory sensilla. These proteins

contain six cysteines with conserved spacing that is the defining feature of the family (Vogt *et al.* 1991). A separate set of secreted proteins with four cysteines are also present in sensillum lymph of olfactory sensilla in Drosophila that are generally considered to be odorant-binding proteins, but there is no functional evidence these proteins bind odorants and they are not considered further here. In Drosophila, seven members of the OBP family have been identified by the use of molecular approaches as genes expressed exclusively in the antenna (McKenna *et al.* 1994; Pikielny *et al.* 1994; Kim *et al.* 1998). More than one OBP member can be expressed in the same sensillum (Hekmat-Scafe *et al.* 1997). To date, only one mutant defective for an odorant-binding protein has been described in any species. Mutants defective for expression of the Drosophila OBP LUSH have abnormal short- and long-term olfactory behavioral responses to a subset of odorants including ethanol, propanol, butanol, and benzaldehyde (Kim *et al.* 1998; Wang *et al.* 2001). These defects are completely reversed by introducing a wild-type copy of the *lush* gene into the mutant animals (Kim *et al.* 1998; Wang *et al.* 2001). These data demonstrate that OBPs perform an important odor-specific function in insect olfaction, but the biochemical role these proteins play is unknown. Other members of this family have been shown to bind directly to odorant ligands with chemical specificity (Vogt and Riddiford 1981; Du and Prestwich 1995; Wojasek and Leal 1999; Sandler *et al.* 2000), indicating these proteins function by specific interactions with subsets of odorant molecules. Models for OBP function include increasing odorant solubility, protection of odorants from enzymes in the sensillum lymph prior to activating olfactory neurons, and removal of odorants from the lymph following neuronal activation (Pelosi 1995; reviewed in Kaissling 2001). Members of the OBP family have not been previously identified in sensillum lymph of any Drosophila gustatory organs.

Adult sensilla arise from sensory mother cell (SMC) precursors during pupal development. The mechanisms required for specification of olfactory and gustatory sensilla are poorly understood (reviewed in Ghysen and Dambly-Chaudiere 1993). However, one putative transcription factor, *poxneuro*, is clearly important for cell fate decisions in gustatory sensilla. *poxneuro* is a paired domain gene product expressed in SMCs of gustatory sensilla. Gustatory sensilla on the legs, wings, and labellum are transformed into the mechanosensory bristle phenotype in mutants defective for *poxneuro* (Awasaki and Kimura 1997). Conversely, misexpression of *poxneuro* in mechanosensory bristle precursors converts mechanosensory bristles to a chemosensory bristle phenotype (Nottebohm *et al.* 1992, 1994). The sensillum transformation in the *poxneuro* mutant appears to include the cell fates of the neurons within these sensilla. Expression of several putative gustatory receptors is lacking in the labellum of *poxneuro* mutants, suggesting the neurons in these sensilla have altered cell fates and no longer express gustatory receptors (Clyne *et al.* 2000).

Understanding how odorant-binding proteins influence olfactory behaviors is an important question in insect olfaction. Toward this goal we have set out to identify all genes encoding members of this protein family in Drosophila. We report here the identification of a large family of putative OBP genes in the Drosophila genome, including a subset expressed exclusively in subsets of gustatory organs. In support of the notion that these gustatory OBPs are expressed in cells required for normal taste function, we show that genetic ablation of cells expressing a leg-specific OBP reduces the *proboscis* extension reflex to sucrose. Finally, we show that Drosophila OBP genes expressed in pharyngeal gustatory sensilla are still expressed in the homeotic mutant *poxneuro*, but gustatory sensilla located on the labellum are not, suggesting that different developmental mechanisms determine cell fate in different taste organs. Our findings imply a broader role for the OBP family in chemical senses in Drosophila and provide new insights into the developmental mechanisms that specify gustatory organ development.

MATERIALS AND METHODS

Fly stocks and transgenic lines: Genetic crosses were carried out under standard laboratory conditions using balancer stocks (Lindsley and Zimm 1992). Drosophila transformations were carried out as essentially as described (Karess and Rubin 1984). Transposase DNA was used at a concentration of 0.25 mg/ml and sample DNA at 0.5 mg/ml. w^{1118} embryos 0–2 hr were injected through the chorion. A minimum of two independent transgenic lines was generated for each OBP promoter fusion to rule out position effects on LacZ reporter gene expression. $poxneuro^{70\text{-}23}$ mutant flies were the generous gift of Dr. K.-I. Kimura (Iwamizawa, Japan). Transgenic flies carrying Grim regulated by upstream activation sequences (UAS) were the gift of John Abrams (Southwestern Medical Center).

DNA, RNA, sequencing, and PCR: Promoters for individual OBP genes were isolated by PCR with primers based on genomic sequence upstream of the predicted coding sequence (see below). In all cases either a *Bam*HI or *Bgl*II restriction site was introduced in the reverse primer immediately downstream of the predicted starting methionine so that the β-galactosidase reporter gene was fused in frame to the first amino acid of the OBP gene. Typically, 3 kb of upstream sequence was isolated but in one case (OBP56h) internal *Bam*HI and *Bgl*II sites were both present in the promoter, so a 1.8-kb fragment was used. A similar-sized promoter fragment faithfully reproduces the LUSH gene expression pattern (Kim *et al.* 1998). A *Not*I restriction site was introduced into the upstream primer to facilitate directional cloning into Casper nls-LacZ. OBP promoters were digested with *Not*I and *Bam*HI (or *Not*I and *Bgl*II) and ligated into Casper nls-LacZ digested with *Not*I and *Bam*HI. Casper nls-LacZ was generated by ligating nls-LacZ (Smith *et al.* 1991; Stamnes *et al.* 1991) as a *Bam*HI-*Sal*I fragment into Casper4 (Pirrotta 1988) digested with *Bam*HI and *Xho*I.

PCR reactions to isolate promoter sequences were performed for 40 cycles of 94° 30 sec, 65° 30 sec, and 72° 2 min using Drosophila genomic DNA for template. All promoter fragments were subcloned into PCR2.1 using TOPO TA (Invitrogen, San Diego) and sequenced to confirm their identity.

Primer sequences for isolation of OBP promoters are as follows:

18a:
5′ GCGGCCGCGCTGCGTTATTTGTTTTATCGT
5′ GGATCCATGGCGAAAATCTGTTTCCCAACT
19a:
5′ GCGGCCGCCCACCTGCGAAATGGGTCATAGTATATGTA
5′ GGATCCATTTCCGAGACGATTTGGCGGATTCCAGA
19b:
5′ GCGGCCGCATTGCTGACGGGTCGAATGGGTCGGAGCGG
5′ GGATCCATTCGGCTGCACTGCATCATTTTTGCTCT
19c:
5′ GCGGCCGCACTGATGGTGTAAAACAAAAAATATGTAAC
5′ GGATCCATTAGCGGAATGGCTGCGACTGGAGTGGA
22a:
5′ GCGGCCGCGTGTCGGGGAACTTTTCCTCAATCCGTCAC
5′ GGATCCATCTCGAAGATCGTTTTTGTTTGCAGCTT
47a:
5′ GCGGCCGCTGCCACAACTTCATTCCCGACTGTCTCGCT
5′ GGATCCATTTTGTTAAATTCGAATGCTTTTATCTG
51a:
5′ GCGGCCGCCGTAAGTTAATATGACTTGTAGCACGATGA
5′ GGATCCATTTTGAGAACTACTAACGATCATGATTA
56a:
5′ GGGCGGCCGCCGATAAAAGGACTTGTGTTCATGTGTGTATGC
5′ GGGAGATCTACGAAGTAGGAGTTCATGTTGAGAAATACTTTGAC
56b:
5′ CCCGCGGCCGCGGCTATTGGCAATCCACTGATGCATGAC
5′ CCCGGATCCAAGTAGATAAGTTTCATCTTTCCAAAGCTAC
56c:
5′ GCGGCCGCTCTGTAAATGTTTTGAATAATAAACTCAAG
5′ AGATCTTTCATGTTCAGCGAGACGGAAAGCGATCGGGT
56d:
5′ GCGGCCGCCTATCTTTAGCAAATAGGCCACTATATTTA
5′ GGATCCATTTTCGGTAGAGATGTTGTTGGAAACCCTT
56e:
5′ GCGGCCGCTTGGAAGTGCAACAGAAGAGTTGATTATTT
5′ GGATCCATGATGCTGATCGTAATCTGCTGTGTAGAAT
56f:
5′ GGGCGGCCGCGTGCAAGTCAGTATTCGATG
5′ CCGGATCCATAATGATAGTTTTGTGTGCAA
56g:
5′ GCGGCCGCCAGCAATTGATGATGGTCTAAGACCCAAGA
5′ GGATCCATTTTAGTTCACTTTTTCGTTTACTAATC
56h:
5′ GCGGCCGCACGGTCTTCGGCTATTCCTAATATCAGTTG
5′ GGATCCATTTTGAGGTATATATTTGTTAAAGCTGT
56i:
5′ GCGGCCGCATCGGACTCGCCCACAGGATATACATAC
5′ AGATCTTTCATTTTTCAGCAGGGTAGGTCATACATGTA
57a:
5′ GGGCGGCCGCTTTTACTAGTTCTCCTTTTG
5′ CCGGATCCATTGTTAACTTCAGACTGAACA
57b:
5′ GGGCGGCCGCGAACTATATCACGTGTAGGT
5′ CCGGATCCATTGTAGAAATGAAACTAAACA
57c:
5′ GCGGCCGCGTCAGTTAATAGTTCTGCCTTTGGGCCAAC
5′ GGATCCATTATCTAACGATTCGCAGAATCTGTTTC
57d:
5′ GCGGCCGCTTAATACGAGTATATCCCAGCAAAATCGAT
5′ GGATCCATTTTTTCAGGCATCAAACTAGTTGAAGA
57e:
5′ GCGGCCGCGGATTTCAGACTGGCAGTAGCTCTCTGGCT
5′ GGATCCATACTTGCTATATTCCTAGGGAATCCATC
83c:
5′ GGGCGGCCGCAAAAACTGAACCGAACTGAA
5′ CCGGATCCATTGCTAAACAATTCTCAATAT
83d:
5′ GCGGCCGCGCGCCCTGGCAGAAACTGGGTTATAACCTT
5′ GGATCCATAAATAGTAATATTTAAAAGCAT
83f:
5′ GGGCGGCCGCCTGCCAAGTCATCTTCATGC
5′ CCGGATCCATCTCTCTGCGGGCAATGCACA
83g:
5′ GCGGCCGCAATTTTATTGTTTAGTTTGCTGGCCGGGCA
5′ GGATCCATTTCTGGCTCGGACGAGGGCTCAAGTGC
99a:
5′ GCGGCCGCGGCCAGGTGACTTGTAATTGTATGTGAGGG
5′ AGATCTTTCATTTTCACTTTCTTTCCACCTATGTATGT
99b:
5′ GCGGCCGCGACGAACCCACTTGACCCATAG
5′ GGATCCATGCTGATGTATGTTTACCTTGTC

The Gal4-VP16 transactivator fusion was made by fusing a Gal4-VP16 encoding sequence with Drosophila translational start and polyadenylation signals. Briefly, the DNA binding domain of Gal4 (amino acids 1–74) fused to VP16 was the gift of Makoto Makishima and David Manglesdorf (Southwestern Medical Center, Dallas). This fusion was cloned by PCR using primers that added a consensus translational start site, an upstream *Xba* site, and an *Nhe* site downstream of the termination codon. This PCR product was sequenced to rule out PCR errors. An SV40 polyadenylation site was added to the 3′ end of this fusion as an *Nhe*I-*Pst* fragment, and the construct was cloned into Casper4 as an *Xba*I-*Pst*I fragment to generate Casper Gal4-VP16. OBP promoters were cloned into the *Not*I-*Bam*HI site of Casper Gal4-VP16.

β-Galactosidase expression: β-Galactosidase was detected in adult heads and bodies as described in Kim *et al.* (1998). Detection of β-galactosidase in larvae was performed as for adult tissues except that the larva cuticle was cut to allow for permeation of fixatives and staining solutions.

Proboscis extension reflex assay: The proboscis extension reflex (PER) was assayed essentially as described by Kimura *et al.* (1986). Briefly, 0- to 2-day-old flies were transferred to a humidified chamber in the absence of food for 16 hr. The flies were immobilized on their backs to the lid of a petri plate using myristic acid. Each fly was allowed to drink water to saturation prior to testing. One prothoracic leg was tested for each fly with a small drop of sucrose solution applied to the

tarsi, and the presence or absence of the PER was noted. Each fly was tested only once on one leg at a single sucrose concentration to avoid potential problems associated with desensitization. The response was recorded as an all or none response. Five sets of 10 flies were tested for each sucrose concentration and each genotype. Significant differences between the means were determined by a two-tailed *t*-test.

RESULTS

Identification of additional members of the Drosophila OBP family: Seven members of the Drosophila OBP family have been previously identified (McKenna *et al.* 1994; Pikielny *et al.* 1994; Kim *et al.* 1998). With the exception of PBP2, which is secreted by epithelial cells (Park *et al.* 2000), each is expressed in the sensillum lymph of a subset of olfactory sensilla (McKenna *et al.* 1994; Pikielny *et al.* 1994; Kim *et al.* 1998; Park *et al.* 2000). How large is the OBP family, and is OBP function restricted to olfactory sensilla? To address these issues, we set out to identify all OBP genes encoded in the Drosophila genome. We have identified 28 new candidate OBP genes in the Drosophila genome project, making a total of 35 members of this gene family present in the genome of this organism. Each putative member was identified with the tBLASTn algorithm (Altschul *et al.* 1990) using the previously identified members of the Drosophila OBP family as probes to identify related genes in the Drosophila genome sequence (Adams *et al.* 2000). Candidate genes were screened for features characteristic of the invertebrate OBP family, including low molecular weight (13–16 kD), a predicted signal sequence, and the presence of six conserved cysteines with the invariant spacing between cysteines 2 and 3 (three residues) and cysteines 5 and 6 (eight residues) that define this family (Vogt *et al.* 1991). Putative genes meeting these criteria typically are predicted to contain one or two introns with conserved splicing consensus sequences (Mount *et al.* 1992) and consensus translation start sequences (Cavener 1987). Each putative OBP member is named with a number representing its chromosomal location and a letter that designates its order relative to other OBP genes at that position. This system is analogous to the nomenclature system used to designate putative odorant receptors (Warr *et al.* 2000). Some OBP genes have been identified by the genome project consortium, but most of the genes reported here were not identified or are predicted to en code different gene products. Only OBP56a and OBP99a were identified as transcribed genes on the basis of expressed sequence tag data and these genes are widely expressed on the basis of LacZ expression (Table 2). The gene structure of several of the OBPs was confirmed by sequencing RT-PCR products (data not shown). It is worth noting that 35 OBP genes is a minimum number, as our analysis would miss genes with introns located between conserved cysteine motifs. It is possible, therefore, that additional OBP genes may be encoded in the Drosophila genome that were not identified here. The genes reported here have been independently identified by Hugh Robertson (personal communication).

OBP genes are clustered in the genome: The genes encoding the OBP family in Drosophila are not randomly distributed in the genome and are often found in large clusters. For example, 14 OBP genes are clustered within 825 kb located at position 56-57 on the second chromosome. A second cluster of 7 genes is located at position 83 on the third chromosome. The localization of so many members of the OBP family in a relatively discrete region of the genome suggests that these genes may have arisen by tandem duplication. Tandem duplication has been suggested to account for the two closely related genes, OBPs 83a and 83b (Hekmat-Scafe *et al.* 2000). Several of the OBP genes are tightly linked. For example, OBP56a and OBP56b are located within 2 kb of each other, but are transcribed in opposite directions. Despite this close proximity, these genes appear to have strikingly different expression patterns (see below). This situation is similar to that observed for clustered putative odorant receptor genes, where closely linked, chemosensory-specific genes are expressed in different olfactory organs (Clyne *et al.* 1999).

Sequence similarity among members of the Drosophila OBP family: The 35 members of the Drosophila OBP family range from 9.2 to 62.6% amino acid identity, demonstrating the highly divergent nature of this protein family. Indeed this protein family is among the most diverse described. Figure 1 shows an alignment of the predicted Drosophila OBP amino acid sequences with the *Bombyx mori* pheromone-binding protein, a moth member of the OBP family. The α-helixes identified by X-ray crystal structure of the *B. mori* pheromone-binding protein are depicted above the alignment (Sandler *et al.* 2000). Intron positions within the Drosophila OBP genes are marked with vertical lines. Most frequently there is a single splice immediately downstream from the DNA encoding the signal sequence. There is little or no amino acid sequence conservation among the OBP members at splice junctions. Several of the OBP genes have unique splicing patterns. Three OBP genes have no splices within the coding sequence (OBP28a, 69a, and 83d). This surprising lack of splicing conservation and low sequence similarity suggests that if gene duplication is the mechanism responsible for the large number of these genes, they evolve rapidly. All members are predicted to encode proteins with six α-helical domains joined by loops that vary in length. Only the six cysteines are completely conserved among all 35 members. The limited sequence similarity among members is often clustered near the cysteines. For example, all members have a hydrophobic amino acid following cysteines two, three, and six. Overall, however, there is little homology in this OBP family. This diversity is con-

FIGURE 1.—Alignment of the Drosophila OBP family with the *B. mori* pheromone-binding protein. Rectangles above the alignment represent α-helical domains identified in the *B. mori* pheromone-binding protein by X-ray crystallography (SANDLER *et al.* 2000). Disulfide bridges, based on the pheromone-binding protein (LEAL *et al.* 1999; SCALONI *et al.* 1999), are predicted to form between the first and third cysteines, the second and fifth cysteines, and the fourth and sixth cysteines in each member. These conserved cysteines, present in all members, are shaded. Vertical lines within the sequences depict splice junctions.

sistent with the notion that different OBPs interact with distinct sets of chemical ligands.

Spatial and temporal expression patterns of the OBP members: To determine the spatial and temporal expression pattern of each putative OBP gene, we fused several kilobases of upstream regulatory sequence for each OBP gene to a reporter gene encoding a nuclear-localized β-galactosidase (see MATERIALS AND METHODS). Transgenic flies were generated that are expected to express the reporter gene in the same cells that normally express that particular OBP. The advantages of this approach over *in situ* hybridization analysis are that it can detect expression in tissues not amenable to *in situ* hybridization (like the wing and legs) and it has little background and excellent sensitivity. Similar fusions have been shown to precisely reproduce wild-type gene expression (SMITH *et al.* 1991; STAMNES *et al.* 1991; TALLURI and SMITH 1995; KIM *et al.* 1998).

We generated transgenic flies carrying reporter constructs fused to each OBP promoter and stained the

TABLE 1

Expression of LacZ regulated by OBP promoters in adult and larvae

	Larvae		Adult	
	Olfactory	Gustatory	Olfactory	Gustatory
19a	−	−	+	−
19b	−	−	−	+
19c	+	−	−	+
19d (PBP2)	ND	ND	+	+
28a (PBP5)	+	−	+	−
56b	+	+	−	+
56c	+	−	+	+
56d	+	−	+	+
56e	−	−	+	+
56g	+	+	−	+
56h	+	+	+	+
57b	−	−	+	+
57c	+	−	+	+
57d	−	−	−	+
57e	−	−	−	+
69a (PBP1)	ND	ND	+	−
76c (lush)	+	−	+	−
83a (OS-F)	−	−	+	−
83b (OS-E)	−	−	+	−
83c	−	−	−	+
84a (PBP4)	ND	ND	+	−
99a	+	−	−	−
99b	−	−	+	−

ND, not determined.

flies for β-galactosidase activity. Table 1 summarizes the expression patterns for several of these new OBP genes on the basis of their expression in olfactory and gustatory expression in adult and larvae. Surprisingly, in addition to the expected olfactory expression, a wide variety of gustatory organs were labeled in many lines. On the basis of reporter gene expression, the members of the OBP family can be classified in one of five classes. Class 1 is composed of putative OBP genes (nine members) expressed exclusively in subsets of chemosensory sensilla. Class 2 genes (four members) are expressed exclusively in gustatory sensilla. Class 3 genes (nine members) are expressed in subsets of olfactory and gustatory sensilla. Class 4 (five members) OBP promoters drive LacZ expression in broad areas that include regions that do not contain chemosensory organs. These genes may be functionally related to PB-PRP2, a gene expressed by epithelial cells that may function as a scavenger protein (Park *et al.* 2000). Class 5 (seven members) includes OBP genes in which no LacZ expression was detectable. These genes may be pseudogenes, or the promoter fragments we used may lack essential regulatory elements required for expression. One putative OBP gene, OBP83e, was not analyzed because no appropriate initiation methionine could be identified. This gene may have a large 5′ intron or may be a pseudogene.

Full analyses of the olfactory and gustatory organs that express LacZ for each OBP promoter fusion are shown in Table 2. Surprisingly, only three new class 1 OBP genes were identified—OBP19a, 57a, and 99a. OBP19a is expressed exclusively in a subset of chemosensory sensilla on the third antennal segment. Figure 2A shows an example of a transgenic fly expressing nuclear-localized LacZ under control of the OBP19a promoter. A bilaterally symmetric pattern of LacZ staining is visible in the third antennal segment, but not the maxillary palps or larval chemosensory organs. This expression pattern is consistent with previously reported members of the OBP family that are also expressed in subsets of olfactory sensilla. OBP57a and OBP99b are expressed in subsets of sensilla in both olfactory organs, the maxillary palps, and third antennal segments (data not shown).

Class 3 genes are expressed in both olfactory and gustatory organs. We identified nine members of this class. Figure 2, B and C, shows examples of two class 3 OBPs with LacZ expression primarily restricted to the olfactory organs, but that also are expressed in at least one gustatory organ. Transgenic flies expressing LacZ under control of OBP56d and OBP57c are expressed in all olfactory sensilla including all sensilla on the antenna and maxillary palp. The expression of these OBPs in the antenna is unique because all other previously reported members are expressed in subsets of sensilla. These lines are not identical, however, as OBP56d is also expressed in the wing and tarsal gustatory sensilla and dorsal organ, and OBP56c is expressed in the wing and the larval olfactory organ, the dorsal organ.

Figure 3 shows examples of some gustatory-specific, class 2 OBPs. OBP19c is expressed exclusively in six cells, including two cells in the labral sense organ (LSO; Figure 3, A and B). The LSO consists of nine sensilla that sample the lumen of the pharynx just distal to the oral opening. Three of the LSO sensilla contain chemosensory neurons; the other six are purely mechanosensory (Stocker 1994). OBP19c is expressed strongly in a single pair of bilaterally symmetric cells in the LSO and weakly in a single cell in each ventral cibarial sense organ (VCSO) and dorsal cibarial sense organ (DCSO) in the adult. The larval dorsal organ also stains for LacZ in these transgenic flies (Figure 3C).

OBP56b is also expressed exclusively in the pharyngeal gustatory organs, including two cells in the LSO and two cells in the DCSO. Figure 3, D and E, shows that this expression pattern is very similar to OBP19c in the LSO. These expression patterns are consistent with expression of these OBPs in support cells of gustatory sensilla, although we cannot precisely identify these cells. Figure 3F shows the expression of OBP56b in third instar larvae carrying the promoter fusion. In Drosophila larvae, volatile odorants are detected by neurons that reside in the dorsal organ, while gustatory responses appear to be mediated by neurons in the terminal organ, in chemosensory neurons located in the ventral pits

TABLE 2

Expression of β-galactosidase in olfactory and gustatory organs by OBP promoters

	Larvae			Adult									
							Cibarial organs						
OBP	Terminal organ	Dorsal organ	Ventral pits	Labellum	Antenna	Maxillary palps	LOS	VSCO	DSCO	Wing	Tarsi	Vaginal plate	Class
18a	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	4
19a	−	−	−	−	+	−	−	−	−	−	−	−	1
19b	−	−	−	−	−	−	−	−	−	+	+	−	2
19c	−	+	−	−	−	−	+	+	+	−	−	−	3
19d (PBP2)	ND	ND	ND	+	+	+	ND	ND	ND	ND	ND	ND	4
22a	−	−	−	−	−	−	−	−	−	−	−	−	5
28a (PBP5)	−	+[a]	−	−	+	−	ND	ND	ND	ND	ND	ND	1
47a	−	−	−	−	−	−	−	−	−	−	−	−	5
51a	−	−	−	−	−	−	−	−	−	−	−	−	5
56a	−	−	+	NS	NS	NS	NS	NS	NS	NS	NS	NS	4
56b	+	+	−	−	−	−	+	+	−	−	−	−	3
56c	−	+	−	−	+	−	−	−	−	+	−	−	3
56d	−	+	−	−	+	+	−	−	−	+	+	−	3
56e	−	−	−	+	+	−	−	−	−	−	−	−	3
56f	−	−	−	−	−	−	−	−	−	−	−	−	5
56g	+	+	−	+	−	−	−	−	−		−	−	3
56h	+	+	+	+	+	−	+	+	+	−	−	−	3
56i	−	−	−	−	−	−	−	−	−	−	−	−	5
57a	−	−	−	−	+	+	−	−	−	−	−	−	1
57b	−	−	−	−	+	+	−	−	−	+	+	−	3
57c	−	+	−	+	+	+	−	−	−	−	−	−	3
57d	−	−	−	−	−	−	−	−	−	−	+	−	2
57e	−	−	−	−	−	−	−	−	−	−	+	−	2
69a (PBP1)	ND	ND	ND	−	+	−	ND	ND	ND	ND	ND	ND	1
76c (LUSH)	−	+	−	−	+	−	−	−	−	−	−	−	1
83a (OSF)	−	−	−	−	+	−	ND	ND	ND	ND	ND	ND	1
83b (OSE)	−	−	−	−	+	−	ND	ND	ND	ND	ND	ND	1
83c	−	−	−	+	−	−	−	−	−	−	−	−	2
83d	−	−	−	−	−	−	−	−	−	−	−	−	5
83e	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
83f	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	4
83g	−	−	−	−	−	−	−	−	−	−	−	−	5
84a (PBP4)	ND	ND	ND	−	+	−	ND	ND	ND	ND	ND	ND	1
99a	−	+	−	NS	NS	NS	NS	NS	NS	NS	NS	NS	4
99b	−	−	−	−	+	+	−	−	−	−	−	−	1

Nonspecific expression indicates cuticular staining not associated with chemosensory organs. ND, not determined. NS, nonspecific.

[a] Park *et al.* (2000).

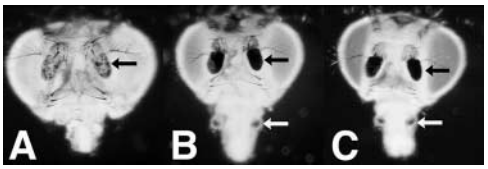

Figure 2.—LacZ expression regulated by the promoters for OBP19a, OBP56d, and OBP57c is exclusively or primarily restricted to the olfactory system. (A) Transgenic flies expressing LacZ under control of the OBP19a promoter indicate that OBP19c is expressed exclusively in a subset of olfactory sensilla in the third antennal segment (arrow). (B) Transgenic flies expressing LacZ under control of the OBP56d promoter express LacZ in all olfactory sensilla on the third antennal segment (black arrow) and maxillary palps (white arrow). Chemosensory bristles on the anterior wing margin and tarsi are also labeled in these flies (see below). (C) OBP57c also drives LacZ expression in all olfactory sensilla. This promoter also expresses LacZ in the labellum (not seen here).

present on each thoracic hemisegment, and in some of the neurons in the dorsal organ. Both the terminal organ and dorsal organs express LacZ in OBP56b-nlsLacZ transgenic flies.

OBP56g is expressed exclusively in the labellum in the adult. Figure 3G reveals that the two outer rows of gustatory bristles are LacZ positive. No other chemosensory organs are labeled in these transgenic flies. The promoter for OBP56h expresses LacZ in approximately five sensilla on each third antennal segment, in the pharyngeal organs (Figure 3I) and in the dorsal organ, the terminal organ, and the ventral pits of the third instar larvae (Figure 3J). This OBP, therefore, may function in both olfactory and gustatory systems.

Some OBP genes are coexpressed in gustatory cells: With the exception of the vaginal plate chemosensory sensilla, we have identified members of the OBP family in all chemosensory organs. Is only one OBP expressed in each gustatory sensillum or can multiple OBPs be coexpressed? Several transgenic lines driving LacZ with different OBP promoters appear to have overlapping expression patterns.

The promoters for OBP57d and 57e drive LacZ expression exclusively in four cells of the tarsi on each of the six legs. Figure 4, A and B, shows expression of β-galactosidase in four cells in the tarsi associated with curved chemosensory bristles in flies expressing nuclear LacZ by the promoter of OBP57d and 57e, respectively. The expression patterns of OBP57d and 57e are also consistent with expression in support cells associated with gustatory sensilla. Interestingly, these genes are located <1 kb apart from each other at the end of the 56-57 gene cluster, have significant amino acid homology to each other in the C-terminal half, and may represent a relatively recent gene duplication event.

Are these OBPs coexpressed in the same cells or different cells that are closely situated? To determine if OBP57d and 57e are coexpressed in the same cells we

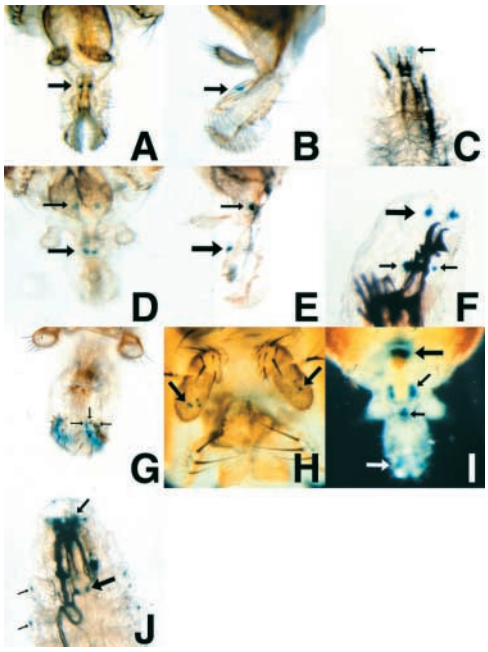

Figure 3.—Expression of LacZ by OBP promoters that are restricted to gustatory sensilla or olfactory and gustatory sensilla. Transgenic flies expressing LacZ regulated by OBP19c (A, B, and C). A and B reveal robust expression in two cells in the labral sense organ (arrow). (C) Transgenic third instar larvae that express LacZ by the OBP19c promoter express β-galactosidase weakly in the dorsal organs (arrow). D and E show frontal and lateral views of the head of a transgenic fly expressing β-galactosidase under control of the OBP56b promoter. The large arrows depict LSO; the smaller arrows depict expression in the VCSO. (F) OBP56b drives LacZ expression in both the dorsal organ (large arrow) and terminal organ (small arrows) in third instar larvae. (G) OBP56g promoter drives LacZ expression exclusively in the labellum. LacZ is expressed in most cells in the outer two of the three rows of taste bristles (arrows). (H, I, and J) The promoter for OBP56h expresses LacZ in a few sensilla on the third antennal segment (H), in the pharyngeal organs (I), and in the terminal organ (large arrow), the dorsal organ (medium arrow), and the ventral pits (small arrows) of the third instar larvae (J).

combined cell-specific ablation with OBP-specific reporter gene expression. The promoter for OBP57e was fused to Gal4-VP16 and transgenic flies were generated that express Gal4-VP16 in the same cells that express OBP57e. Gal4-VP16 is a modified yeast transactivator that binds to UAS sites. These flies were crossed to UAS Grim transgenic flies to make a double homozygous stock. Grim is a proapoptotic factor that results in programmed cell death when misexpressed in Drosophila cells (Chen *et al.* 1996; Wing *et al.* 1999). Therefore, Grim expression is expected to be restricted to the cells expressing Gal4-VP16 regulated by the OBP promoter, and this expression should result in the death of these cells. Flies homozygous for OBP57e Gal4-VP16 and UAS Grim were crossed to flies homozygous for the promoter for OBP57e directly driving expression of LacZ. The progeny of this cross receive a single copy of each trans-

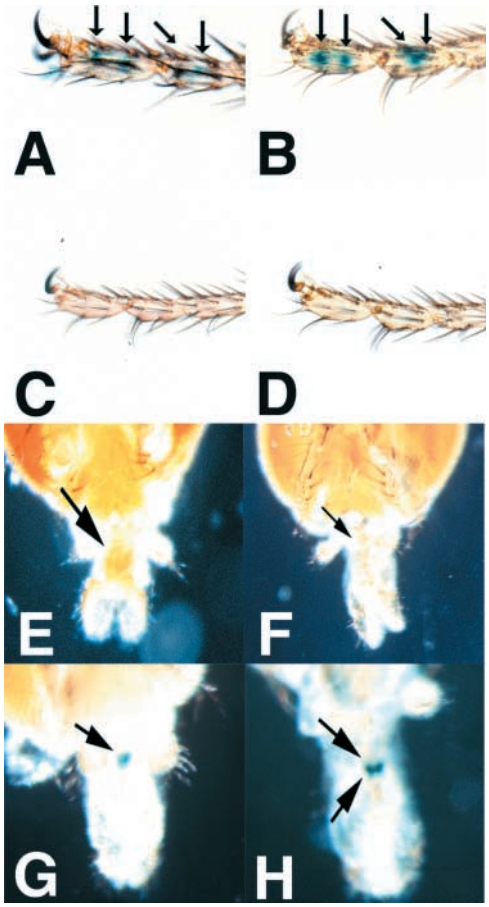

FIGURE 4.—Promoters for OBP57d and 57e are expressed exclusively in four cells associated with chemosensory bristles on each leg. (A) OBP57d promoter drives LacZ in four cells beneath tarsal taste bristles (arrows). (B) OBP57e promoter expresses LacZ in a similar pattern. (C) Transgenic flies expressing both the cell death gene Grim and LacZ under control of the OBP57e promoter. Expression of the Grim transgene eliminates LacZ expression. (D) Expression of Grim by OBP57e eliminates staining of cells expressing LacZ under control of the OBP57d promoter. (E) Flies expressing Grim and LacZ under control of the OBP56b promoter lack LacZ expression, demonstrating the ablation of the OBP56b-expressing cells. Arrow indicates location of the LSO. (F) Flies expressing Grim under control of the 56b promoter and LacZ under control of the 19c promoter also have no detectable LacZ expression. (G) Flies expressing Grim under control of the OBP56b promoter and LacZ under control of the 56h promoter still have LacZ expression in the LSO. (H) Flies expressing LacZ under control of both the 56b and 56h promoters display broader LacZ expression.

gene, allowing us to assay the effectiveness of Grim killing the OBP57e-positive cells. As shown in Figure 4C, expression of LacZ driven directly by the OBP57e promoter is not detectable. These results indicate that the cells expressing OBP57e are ablated. Is OBP57d expressed in the same cells as OBP57e? We crossed the pOBP57e Gal4-VP16; UAS Grim flies to flies expressing LacZ directly under control of the OBP57d promoter. Figure 4D shows that ablation of the cells expressing OBP57e also eliminates LacZ expression driven by the promoter for OBP57d. These data indicate that OBP57d and 57e are coexpressed in the same cells in the tarsi.

The promoters for three OBP genes, OBP56b, OBP56h, and OBP19c, drive LacZ expression in single pairs of cells in the pharyngeal LSO. Are any of the OBPs expressed in the LSO coexpressed in the same cells? To determine if OBP19c and 56b and 56h are coexpressed, we used the strategy described above. We used the promoter for OBP56b to drive expression of Gal4-VP16 (see MATERIALS AND METHODS) and crossed these flies to transgenic flies carrying UAS Grim. We made a stock of flies homozygous for both pOBP56b-Gal4-VP16 and UAS Grim transgenes. To confirm that the cells that normally make OBP56b are ablated in this stock, we crossed the double transgenic flies to flies carrying the OBP56b promoter directly driving nuclear-localized LacZ. The progeny of this cross fail to express LacZ in the LSO, indicating these cells have undergone apoptosis (Figure 4E). If OBP56h and OBP19c are coexpressed in the same cells as OBP56b, then LacZ expressed directly by the OBP56h or 19c promoters should also be absent in the pOBP56bGal-VP16;UAS Grim background. By contrast, if these OBP promoters drive expression of LacZ in closely opposed, but different cells, expression of LacZ will not be ablated. We crossed each of these promoter fusions directly driving LacZ to the double transgenic flies expressing Grim in the OBP56b cells. The progeny from the OBP19c cross have no LacZ expression in the LSO (Figure 4F). This result indicates that these two OBP genes are coexpressed in the same cells. However, progeny from the cross to the OBP56h promoter directly driving LacZ to the double transgenic flies have LSO cells that robustly stain for LacZ (Figure 4G). These results indicate that OBP19c and OBP56b are coexpressed in the same LSO cells, but OBP56h is expressed in neighboring cells that do not express OBP56b. Indeed, when the two reporter strains expressing LacZ under control of the OBP56b and 56h promoters are crossed together, broader LacZ expression is apparent (Figure 4H).

The proboscis extension reflex is eliminated in flies lacking the cells that make OBP57e: We used the transgenic flies expressing Grim exclusively in the OBP57e-positive cells in the tarsi to evaluate the biological importance of these cells in gustation. We tested wild-type and pOBP57eGal4-VP16;UAS Grim-expressing flies for their ability to detect sucrose applied to the tarsi using the PER. Normally when sucrose is applied to the tarsi, the fly extends the proboscis to feed (KIMURA *et al.* 1986). Figure 5 shows that expressing Grim in the cells expressing OBP57d and 57e results in a dramatic loss of sensitivity to sucrose as determined by this assay. Wild-type control flies have strong PERs to sucrose concentrations as low as 10^{-6} M sucrose. However, flies lacking the cells that express OBP57d and 57e have dramatically reduced PERs to sucrose concentrations from 10^{-6} to 10^{-2} M.

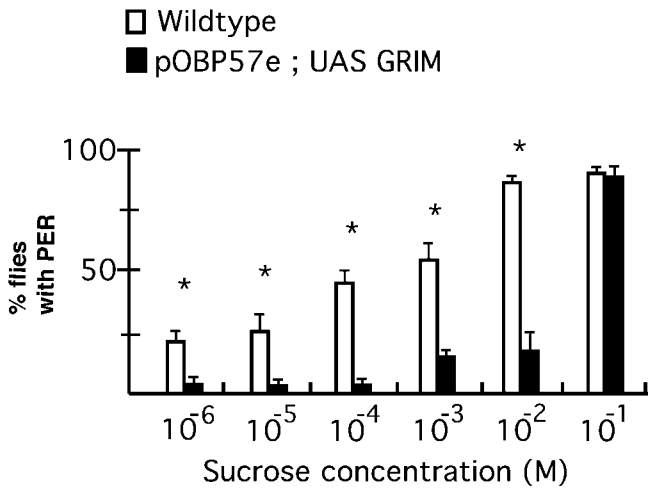

FIGURE 5.—The proboscis extension reflex to sucrose is abnormal in transgenic flies expressing the cell death gene *Grim* in the cells that make OBP57e. Flies extend their proboscis in response to application of sucrose to the terminal tarsi of the prothoracic legs (see MATERIALS AND METHODS). Flies expressing Grim in the tarsi cells that express OBP57e have a dramatic reduction in their sensitivity to sucrose. However, responses of the Grim-expressing flies and wild-type controls to 100 mM sucrose (10^{-1}) are not different. Error bars represent standard error of measurement. Asterisk denotes statistically significant differences between the two groups by *t*-test.

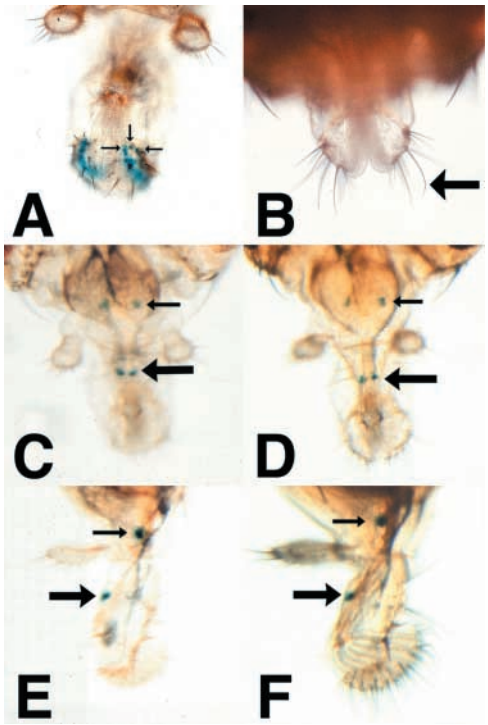

FIGURE 6.—*poxneuro* mutants express LacZ regulated by OBPs expressed in the pharyngeal taste organs but not the labellar taste organs. β-Galactosidase expression regulated by the OBP56g promoter is eliminated in the *poxneuro* genetic background. (A) OBP56g promoter drives LacZ expression in the labellum in the wild-type genetic background. (B) LacZ expression is abolished when the transgene is in the *poxneuro* genetic background. (C and E) LacZ expressed under control of OBP56b reveals β-galactosidase in two cells of the LSO (large arrow) and VCSO (small arrow). (D and F) β-Galactosidase expression is unchanged in mutants homozygous for *poxneuro*.

Interestingly, at concentrations of 10^{-1} M, there is no difference in the probability of eliciting the PER between the two groups. This demonstrates that the cells that normally express OBP57d and 57e are important for normal sensitivity of the tarsi to sucrose, but at high sucrose concentrations the loss of these cells has no effect. Therefore the PER reflex is intact in the Grim-expressing animals, but the sensitivity to sucrose is blunted. These results confirm that the cells expressing OBP57d and 57e are required for normal gustatory sensitivity of the tarsi to sucrose.

***poxneuro* eliminates OBP expression in labellar but not pharyngeal gustatory sensilla:** *poxneuro* is a paired domain transcriptional regulator. Mutants defective for *poxneuro* have an abnormal number of leg segments and conversion of labellar gustatory sensilla to mechanosensory bristle phenotype (AWASAKI and KIMURA 1997). RT-PCR experiments reveal that *poxneuro* mutants fail to express most, but not all, putative gustatory receptors (CLYNE *et al.* 2000). To determine if *poxneuro* transforms the support cells that make OBPs, we determined whether reporter genes regulated by the promoter for OBP56g are still expressed in the *poxneuro* mutant background.

Figure 6A shows the LacZ expression in labellar sensilla in wild-type flies expressing LacZ under control of the OBP56g promoter. When the *P* element carrying this construct is crossed into the *poxneuro* genetic background, LacZ expression is completely absent in the labellum (see Figure 6B). These results indicate that the cells in labellar sensilla do not express OBP56g in the *poxneuro* genetic background. This supports the notion that *poxneuro* acts early in the development of chemosensory sensilla to delegate chemosensory identity on all cells in the sensillum, including the cells that synthesize and secrete OBP56g.

To assess the role of *poxneuro* in the differentiation of the pharngeal chemosensory organs, we crossed flies carrying the OBP56b promoter driving LacZ expression in specific pharyngeal organs into the *poxneuro* genetic background. Figure 6, C and E, shows LacZ expression regulated by OBP56b in a wild-type genetic background, and Figure 6, D and F, shows the same construct in the *poxneuro* genetic background. *poxneuro* does not disrupt expression of LacZ regulated by the pharyngeal OBP promoter OBP56b. Together, these data indicate that *poxneuro* is required for expression of OBP56g in the labellar gustatory sensilla, but not for OBP56b expressed in pharyngeal gustatory organs. This suggests that different developmental mechanisms are required for the proper specification of pharyngeal and labellar gustatory sensilla.

DISCUSSION

Size and diversity of the family: We report here the identification and expression of a large family of putative odorant-binding protein genes in *D. melanogaster*. Twenty-eight new members have been identified, only three of which are expressed in subsets of olfactory sensilla like previously identified members of the family. Therefore, the molecular and genetic screens performed to identify antenna-specific molecules successfully identified most, but not all, members of this family with expression restricted to the olfactory system. Other members are expressed in a wide range of chemosensory organs and would not be identified in screens designed to recover antenna-specific genes. Because of the diversity of the family and the expression of this family in most, if not all, chemosensory sensilla it is unlikely that most of these genes would have been identified in the absence of the genome sequence.

Two features of this gene family are extraordinary: the low degree of sequence similarity among the family members and the location of so many members in large gene clusters. The diversity of the family based on amino acid homology is striking. Only the six conserved cysteines are conserved in all members, probably reflecting a requirement for proper disulfide bonding for functional tertiary structure. Most of the genes have a single splice junction located after the signal sequence. This may reflect a common ancestor for many of these genes. However, many genes have two splices, and often these occur in novel positions. Other than immunoglobulin gene families that underwent an explosive increase in number and diversity in early jawed vertebrates, the other large gene family that has undergone rapid diversification is another chemosensory-specific gene family, the odorant receptors. Seven transmembrane receptor families mediate chemical detection in vertebrates, *Caenorhabditis elegans*, and Drosophila (Buck and Axel 1991; Troemel *et al.* 1995; Clyne *et al.* 1999, 2000; Gao and Chess 1999; Vosshall *et al.* 1999). These mechanisms could have arisen independently in the three animal groups or, more likely, evolved from a common ancestor early in the animal lineage and diverged. While there is little or no sequence similarity between the receptor families in these three groups of animals, there are similarities between receptor genes within an organism, indicating that diversity within an organism may arise by gene duplication. The OBP family described here has little similarity among the Drosophila members. The presence of most of these genes in clusters suggests these genes arose by tandem duplication. Almost half of the OBP genes are located within 825 kb of genomic DNA located at chromosomal position 56-57 on the right arm of the second chromosome. This clustering is consistent with a gene duplication mechanism for generating the large size of the gene family. OBP83a and OBP83b are closely related and juxtaposed in the Drosophila genome, but there is only a single related gene present in other Drosophila species (Hekmat-Scafe *et al.* 2000). This strongly supports a gene duplication mechanism accounting for the size of this family. However, the low sequence identity among the members suggests that there is rapid evolution of these sequences following duplication. Perhaps the OBP genes are evolving more rapidly than the receptor family. If true, this could reflect constraints on the evolution rate of the receptor family, which must interact with other signaling molecules, or there may be unappreciated positive selective pressure to have a diverse OBP population.

The pheromone-binding protein from the moth *B. mori* undergoes pH-dependent conformational changes thought to reflect a mechanism for loading and unloading the pheromone (Wojasek and Leal 1999). In particular it has been suggested that two adjacent histidines present in several pheromone-binding proteins just before α-helix 4 may be important for these conformational changes and, thus, for the function of these proteins. If this is true, this feature should be conserved among other members of the family. When the Drosophila proteins are aligned with the *B. mori* pheromone-binding protein (Figure 1), we find that these histidines are not conserved in the Drosophila sequences. In fact none of the 35 predicted Drosophila proteins contain this motif, although some putative proteins have histidines in this general vicinity. Either the moth pheromone proteins function differently from the other OBP family members or the conformational changes suggested to be important for loading and unloading ligands do not specifically require these residues.

Role in olfaction and gustation: The basis for odorant and tastant discrimination in Drosophila is unknown, but probably is mediated by two gene families, seven transmembrane receptors, and odorant-binding proteins. Recently, a family of ~70 Drosophila genes were discovered that encode seven transmembrane receptors expressed in subsets of olfactory neurons [the olfactory receptors (ORs); Clyne *et al.* 1999; Gao and Chess 1999; Vosshall *et al.* 1999]. Neurons expressing the same receptor converge to a single glomerulus in each antennal lobe (Gao *et al.* 2000; Vosshall *et al.* 2000), the insect equivalent of the olfactory bulb (reviewed in Hildebrand and Shepherd 1997). Convergence of neurons expressing the same odor receptor is also observed in vertebrate olfactory systems (Ressler *et al.* 1994; Vassar *et al.* 1994) and suggests that the Drosophila neurons expressing the same receptor are functionally related. However, a direct role for these ORs in olfaction has yet to be demonstrated.

A second family of genes encoding seven transmembrane receptors that encode putative gustatory receptors has been identified (GRs; Clyne *et al.* 2000). Some of these GR genes are expressed in subsets of gustatory neurons in adults and larvae, consistent with a role in

mediating taste transduction, but other members are expressed in subsets of olfactory neurons (Scott *et al.* 2001). If these OR and/or GR gene families mediate chemosensory responses, it would suggest that the chemical specificity of chemosensory neurons results from the choice of receptor expressed by that neuron. Indeed, mutants defective for the trehalose receptor have abnormal gustatory behavior to trehalose (Ishimoto *et al.* 2000). Are receptors the sole source of chemosensory specificity in insects?

Unlike vertebrate olfactory neurons, insect chemosensory neurons are segregated into separate compartments; therefore access of chemical ligands to the chemosensory neurons can be independently regulated in different sensilla. Expressing chemical-specific components in the sensillum lymph could sensitize the neurons in that sensillum to specific molecules by concentrating them in the lymph or, alternatively, chemical-specific factors in the lymph could act as filters or in ligand removal to reduce the sensitivity of the neurons to specific molecules. In this manner, nonneuronal components expressed in the sensillum lymph could influence the chemical specificity or sensitivity of olfactory neurons within the sensillum. The invertebrate OBPs may play such a role in chemosensory sensilla. Odor-specific defects in the *lush* mutants support the notion that OBPs are important for olfactory behavior and odor discrimination (Kim *et al.* 1998; Wang *et al.* 2001). Indeed, LUSH is the only molecule specifically expressed in the olfactory system that has been proven to influence olfactory behavior in Drosophila.

Are OBP proteins important for gustatory transduction as well? No mutants are available in any of the gustatory OBP genes. However, the expression of most of the OBP genes in gustatory organs supports this idea. Furthermore, ablation of the cells that express OBP57d and 57e in the tarsi results in defective gustatory reflexes mediated through contact chemoreceptors on the tarsi. This supports the idea that the cells that synthesize these OBPs in the tarsi are required for normal chemosensory responses mediated through the tarsi. This defect could arise because the support cells may be required to maintain the neurons in a functional state. Alternatively, the defective responses to sucrose could result from the specific loss of one or both of these OBPs from the sensillum lymph of the tarsi chemoreceptors. This issue could be resolved by generating mutants defective for OBP57d and 57e and determining if they have defective responses to sucrose using the PER. This would differentiate the role of the support cell from the role of the binding proteins themselves. It is intriguing that the response to sucrose is defective in the Grim-expressing flies at low sucrose concentrations, but is normal at 100 mm. This suggests either that the sucrose-responsive neurons are less sensitive in the absence of the support cells or that other sugar-responsive neurons with a higher activation threshold are present that are unaffected.

Recent studies analyzing the expression of members of the OR and GR families suggest that the difference between smell and taste in Drosophila is not based on the receptor family expressed, but on the location and connections of the neurons that express the receptor. Indeed members of the GR family probably mediate olfactory responses (Scott *et al.* 2001). In olfactory and gustatory sensilla, ligands must enter the sensillum lymph to interact with receptor molecules located on the neuronal dendrites. Therefore, in sensilla that mediate both taste and smell the ligand is in solution. Tastants are generally soluble molecules, but many odorants are hydrophobic with low water:oil solubility ratios. Therefore, the role of the OBPs has previously been thought to be odor specific and possibly associated with the special problem of getting odorants into solution. The presence of OBPs in gustatory sensilla forces us to rethink this issue. Indeed, *lush* mutants have defective behavioral responses to ethanol, a molecule that should have little difficulty entering aqueous or hydrophobic environments. Perhaps OBPs function to protect chemicals ligands from enzymatic modification in the sensillum lymph, as a variety of enzymes are expressed in the sensillum lymph, including cytochrome P450 enzymes (Wang *et al.* 1999). Whatever the biochemical role of the olfactory OBPs, it seems likely that this function is important for both olfactory and gustatory physiology.

***poxneuro* and development of gustatory sensilla:** The *poxneuro* mutant is particularly interesting to Drosophila biologists studying taste, as this mutant specifically converts gustatory sensilla in the labellum to a mechanosensory phenotype. Indeed, the loss of receptor expression in *poxneuro* mutants was used to argue that members of the GR family are gustatory receptors (Clyne *et al.* 2000). The nonneuronal support cells that secrete sensillum lymph and the chemosensory neurons are derived from common progenitor cells during development. Therefore, if *poxneuro* functions in the progenitor cell, it is likely that the chemosensory support cells would also be converted to the mechanosensory organ fate in *poxneuro* mutants. Do the transformed bristles in *poxneuro* mutants still express chemosensory-specific OBPs? The LacZ reporter regulated by the promoter region of OBP56g is not expressed in the labellum in *poxneuro* mutants. This indicates that the support cells have undertaken the same mechanosensory fate as their neuronal sisters. By contrast LacZ reporter regulated by OBP56b, expressed in the pharyngeal organs, is still expressed normally in *poxneuro* mutants. This implies that different developmental programs are utilized to differentiate gustatory organs located in different regions.

Overall, our studies support a broad role for members of the OBP family in gustation and olfaction and provide insights into the developmental mechanisms responsi-

ble for the determination of different sets of gustatory sensilla. Future studies will be required to define the developmental mechanisms mediating cell fate determination in pharyngeal gustatory organs and the biochemical roles of the members of the OBP family.

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